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RESEARCH ARTICLE

Popping patterns in F2 segregant progenies from popcorn × non-popcorn crosses

Viridiana Trejo-Pastor[a,b], Amalio Santacruz-Varela[b]*, Higinio López-Sánchez[c], Denise E. Costich[d], Leobigildo Córdova-Téllez[b], Roberto de la Cruz Díaz-Juárez[e].

[a]Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Cuautitlán, Cuautitlán Izcalli, Estado de México, México, [b]Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México, México, [c]Colegio de Postgraduados, Campus Puebla, San Pedro Cholula, Puebla, México, [d]Institute for Genomic Diversity, Cornell University, Ithaca, New York, USA; [e]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Zacatepec, Zacatepec, Morelos, México

ABSTRACT

The most important commercial feature in popcorn (*Zea mays* L.) is the expansion of the starch contained in the endosperm; however, little has been studied about the underlying genetic basis of this quantitative trait, for which the development of segregating populations is required. The first step is the selection of the most appropriate contrasting parents for popping capacity to create these populations. The objectives of this study were, 1) to analyze the popping patterns of F2 populations derived from crosses between popcorn × non-popcorn inbred lines to identify the most divergent cross for future molecular analysis in search of alleles-related to popping capacity, and 2) to estimate the number of genes responsible for popping expansion volume based on the tails of segregation. Seven biparental crosses of popcorn × non-popcorn were performed to obtain F2 populations, and those were phenotyped for popping expansion volume. Phenotypes were analyzed by the H test, Lilliefors normality-test, Fisher's coefficient of skewness, and kurtosis level. The number of genes estimation was made with X^2 and $(1/4)^n$ tests. The popping patterns indicated that popcorn × flint corn was the most divergent cross, with the most symmetrical distribution, and therefore, the best suitable population to be used for molecular analysis. Results indicated the existence of three to five major genes related to popping expansion volume. Transgressive segregation was present in all populations, showing individuals whit a popping expansion volume higher or lower than the parental ones, suggesting that non-popcorn lines contain variation for **alleles that promote popping.**

Keywords: Popcorn, popping expansion volume, popping patterns, transgressive segregation, *Zea mays* L.

INTRODUCTION

The center of origin of popcorn (*Zea mays* L.) is Mexico (Beadle, 1939; Miranda, 1966; Matsuoka et al., 2002; Doebley, 2004), where the current consumption of this type of corn is 66.4 thousand tons per year, of which only 1.5 thousand tons per year are produced in the country (SIAP, 2020). The overwhelming majority (98%) is imported, mainly from USA, and to a lesser extent from Argentina (SIAVI, 2020). The low national production is due to the scarcity of genetic programs to improve the popping capacity of native popcorn races, as exposed in a comparative study about popping expansion volume between Mexican popcorn races and a group of North American Popcorn races, in which an expansion ratio of 1:24 (48.8 vs. 1,166 cm^3 30 g^{-1}) was found in favor of the North American races specialty the North American Yellow Pearl Popcorn (Santacruz-Varela, 2004).

The physical and morphological characteristics of the kernel that contribute to the popping capacity are numerous. They include a more crystalline arrangement of the endosperm, the polygonal arrangement of the starch, pericarp hardness, amylose/amylopectin ratio, a higher degree of fibrillar packing of the pericarp, porosity, moisture content, and the presence of protein bodies in the pericarp and endosperm, especially the α-zeins (Hoseney et al., 1983; Park et al., 2000; Gökmen, 2004; Babu et al., 2006; Borras et al., 2006; Lee et al., 2000; Ertaş et al., 2008; Sweley et al., 2013; Dong et al., 2015; Vázquez-Carrillo et al., 2019).

Likewise it is necessary to consider that the evaluated trait is relatively complex and is affected, in addition

***Corresponding author:**
Amalio Santacruz-Varela, Colegio de Postgraduados, Campus Montecillo, Postgrado en Recursos Genéticos y Productividad-Genética, km 36.5 Carretera México-Texcoco, 56230, Montecillo, Texcoco, Edo. de México. **E-mail:** asvarela@colpos.mx, **Phone:** +52 (595) 95 1620 200 Ext. 1570.

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to the genotype, by other factors such as the popping method used (oil pot, microwave oven, hot air, hot sand), size, volume, and moisture content of the kernel, drying procedure, damage to the pericarp and endosperm, among others (Hoseney et al., 1983; Ziegler, 2001; Allred-Coyle et al., 2002; Gökmen, 2004; Lee et al., 2000; Ertaş et al., 2008), which points towards a multifactorial inheritance, in addition to the fact that there are environmental characteristics that modify the expression of EV; thus, its study deserves further attention.

The methods for the analysis of popping in small samples focus on the use of tools such as microwave ovens (Robins and Ashman, 1984; Ziegler, 2001; Babu et al., 2006; Li et al., 2021) or electric popper (Coan et al. al. al., 2019), because there are often small amounts of grain from a single ear or in populations with few seeds; this protocol is routinely used in popcorn breeding programs.

Popping capacity has been hypothesized to be a quantitative trait with additive genetic effects, conditioned by three to five genes of major effects. However, the studies which postulate that hypothesis do not mention the methodology used to support their results (Ashman, 1983; Ziegler, 2001) other studies mention genomic regions that explain a fraction of the phenotypic variance (Lu et al., 2003; Babu et al., 2006; Dong et al., 2015; Coan et al., 2019), if this hypothesis is verified, the genetic improvement to accumulate favorable alleles for popping capacity in native populations would be relatively straightforward.

An important step in the study of the genetics of popping capacity is the formation of biparental crosses between popcorn and contrasting endosperm lines to obtain segregating populations and use them to determine causal associations between markers and phenotypic traits; however, there is a wide diversity of endosperm that can be used, so it is important to define a type of endosperm that generates a population as divergent as possible from a phenotypic point of view that facilitates selective genotyping and, in general, the study of the quantitative genetics of the trait (Sun et al., 2010; Zou et al., 2016).There are genomic studies that have reported since three QTLs (Li et al., 2006; Paes et al., 2016) to 11 QTLs (Dong et al., 2012) involved in the popping capacity, using biparental crosses between popcorn × dent or flint corn (Lu et al., 2003; Babu et al., 2006; Li et al.,2006; 2007; 2009; Dhliwayo, 2008; Dong et al., 2012; 2015; Paes et al., 2016; Coan et al., 2019). A recent study reported by Li et al. (2021), identified 162 significant SNPs associated with popping-related traits within annotated gene regions based on GWAS, and 118 known genes were mapped by the significant SNPs. However, due to the complex nature of the endosperm and its direct influence on the popping characteristics, the participation of the non-popcorn parent in the results is not clear, so uncertainty persists about the most appropriate non-popcorn parent to obtain a greater phenotypic amplitude among segregating individuals in order to make neater estimates on the number of genetic factors involved in the popping capacity.

Thus, the objectives of this study were (i) to generarte F2 populations from biparental crosses of popcorn × non-popcorn maize in order to identify the influence of different endosperm types on patterns of segregation of the popping expansion volume, (ii) to identify the most divergent cross as an amenable population for future molecular studies at identifying the alleles that contribute to the popping capacity, and (iii) to estimate the number of genes responsible for popping expansion volume based on the tails of segregation patterns.

MATERIALS AND METHODS

Genetic material

Eight CMLs (CIMMYT Maize Lines) adapted to the highlands agroecosystem were used (Table 1), they were provided by the Germplasm Bank of the International Maize and Wheat Improvement Center (CIMMYT) (MGB: http://hdl.handle.net/11529/10246/23). CML561, with the highest popping expansion volume of all the CMLs, was selected as the female parent line and seven lines with non-popcorn endosperm with adaptation to highlands: CML 240, CML 246, CML 459 and CML 527 with semident endosperm, CML457 and CML461 with dent endosperm and CML558 with flint endosperm, were used as male parents (Table1). During the 2015 Spring-Summer cycle (April-November), biparental crosses were formed at the CIMMYT experimental station in Metepec, State of Mexico located at coordinates 19°13'38.6"N and 99°33'01.6"W at an altitude of 2625 masl. One hundred seeds of each line were planted in seven plots of 5.0 m long × 0.8 m wide rows, with a planting distance of 0.30 m, each biparental cross was carried out through artificial plant-to-

Table 1: Inbred lines used to develop seven popcorn×non-popcorn crosses and the F2 segregant progenies

Line	MT	DMF	DFF	K	HG	(cm³ 30 g⁻¹)
CML240	Early	80	86	Semident	A	85
CML246	Intermediate	85	90	Semident	B	98
CML457	Intermediate	88	90	Dent	A	85
CML459	Intermediate	84	89	Semident	B	75
CML461	Early	84	85	Dent	AB	95
CML527	Early	81	86	Semident	B	158
CML558	Late	90	94	Flint	AB	170
CML561	Intermediate	89	91	Popcorn	B	460

MT: maturity category, DMF: days to male flowering, DFF: days to female flowering, K: Kernel type, HG: heterotic group, EV: popping expansion volume.

plant pollination. The F1 crosses, along with the parental lines, were planted during the 2016 Spring-Summer cycle (May-December) at the experiment station of Colegio de Postgraduados (COLPOS-Montecillo) in Texcoco, State of Mexico located at coordinates 19° 27' 26"N and 98° 54' 30"O at an altitude of 2245 meters above sea level, in plots whit 20 rows of 9.0 m long × 0.8 m wide, with a planting distance of 0.30 m. Manual self-pollination was performed to obtain the groups of F2 individuals, which constituted the analysis populations.

Phenotyping of F_2 segregating populations

The F_2 segregating populations and parental lines were planted during the 2017 Spring-Summer cycle at the COLPOS experiment station under the same field conditions as in the 2016 cycle, and once the ears reached physiological maturity, they were harvested with a moisture content of 16 ± 2%. The ears of each F_2 population were stored at room temperature for 30 days; afterward, the samples were formed by taking 30 g of kernels if possible, from the middle part of each ear and weighed with an analytical balance (Ohaus Adventurer®, H-5276, USA). The samples were placed in organza bags in a controlled environment chamber (CONVIRON S10H, Series 970106, Canada) at a temperature of 21 °C and 70% relative humidity for 30 days, where an equilibrium moisture content of 13.5 ± 0.5% was reached (Ziegler, 2001).

Popping capacity was evaluated by measuring popping expansion volume, using the following methodology developed by the Iowa State University Popcorn Program (Ziegler, 2001). The phenotyping protocol is also available at http://hdl.handle.net/11529/10548274.

Statistical analysis

The EV was analyzed through a normality test for a number of > 50 observations, a modification of Kolmogorov-Smirnov method, and the Lilliefors test (L) with a significance level of $P \leq 0.05$. A histogram of the phenotypic frequency distribution was created for each biparental cross to observe symmetry and to detect the population with the widest interquartile range (Sun et al., 2010). A Quantile-Quantile plot (Q-Q plot) was used to visualize the EV distribution and to observe possible deviations from the theoretical normal distribution when the data deviate from the diagonal of the graph. Given the positive asymmetry of the data, the Kruskal-Wallis non- parametric test, or H test, was used as an alternative to ANOVA, using intervals to test the null hypothesis that k samples have been obtained from the same population or from identical populations with the same median. The Mann-Whitney-Wilcox *post hoc* comparison method was subsequently used to estimate the critical value of the H test to obtain the significance level on the segregating populations using the *holm* significance level correction with a significance level of $P \leq 0.05$. The analysis of Kurtosis –the combined weight of the tails with respect to the mean– (K) and the calculation of the Fisher's coefficient of Skewness (FCs) were performed using the R software (version 3.5.3) with the 'agricolae' package (https://CRAN.R- project.org/package=agricolae).

The estimation of the number of genes involved in EV was performed using the chi-square test (X^2) with the phenotypic data from each population. For the X^2 test, n-1 degrees of freedom were used, where n is the number of classification groups of the expected frequencies with a significance level $P \leq 0.05$. Frequencies for expected phenotypic groupings were empirically estimated with the cumulative binomial distribution formula:

$$p^{(k-m)}q^{m} = \left[\frac{m!(k^{k!}-m)!}{C_{k,m}}\right]p^{(k-m)}q^{m}$$

where *C* indicates the number of phenotypes of each type; *p* and *q* indicate the dominant and recessive alleles, respectively; *k* and *m* indicate the number of phenotypic classes. The resolution of the binomial indicates the probability or proportion of each phenotypic class, which made it possible to carry out groupings as follows, G1: homozygous individuals with EV similar to P1; G2: homozygous individuals with EV similar to P2; G3: heterozygous individuals with intermediate EV (García-León et al., 2018). The hypothesis for the X^2 test was formulated on the assumption that popping capacity in popcorn is conditioned by three to five additive genes with the same effect, each under Mendelian segregation of 1: 2: 1, as expected in any F_2 (Ashman, 1983; Ziegler, 2001; Lu et al., 2003; Babu et al., 2006; Coan et al., 2019). For example, if the popping capacity was conditioned by three genes, the expected frequency of homozygous individuals equals to the popcorn parent would be 1/64 (1.6% of individuals) and this would be equal to the expression $(1/4)^n$; where, n represents the estimated number of genes involved in the polygenic trait.

RESULTS AND DISCUSSION

Analysis of EV segregation patterns

Segregation patterns of the F_2 populations of seven crosses of popcorn × non-popcorn, grouped by endosperm type with their respective graphical normality test for EV, are shown in Figs. 1 to 4. The segregation patterns showed differences in regard to the length and weight of the tails as a function of the endosperm type, as well as the

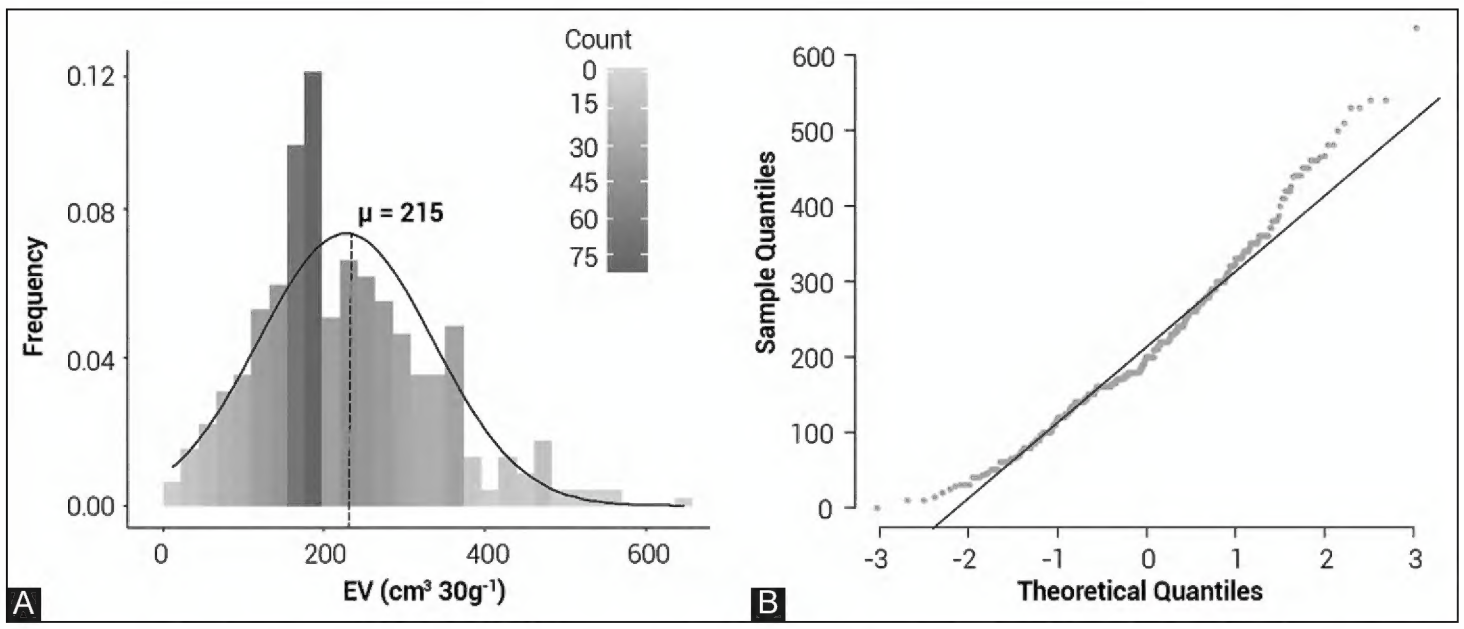

Fig 1. Phenotypic frequencies (A) and Q-Q plot (B) of popping expansion volume evaluated in the F_2 population derived from CML561 (popcorn) × CML558 (flint).

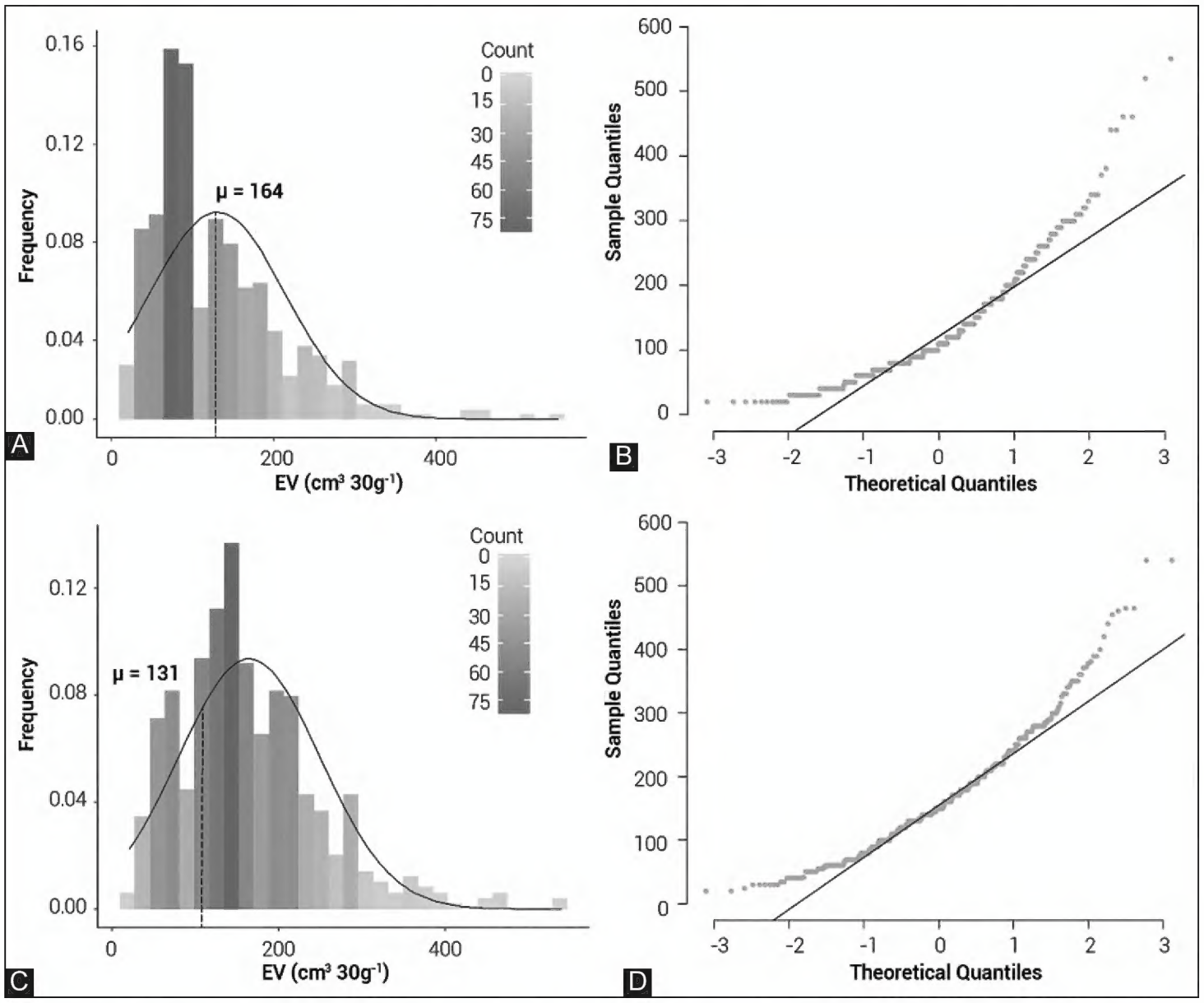

Fig 2. Phenotypic frequencies and Q-Q plot of popping expansion volume evaluated in F_2 populations derived from the crosses between popcorn CML561 and dent CML461 a, b), and CML457 c, d).

presence of extreme individuals, superior to the parental; that is, transgressive segregation was observed, induced by the meiotic recombination phenomenon thus makes possible the combination of favorable or not alleles showing F2individuals whit EV higher or lower than the parental ones.

In regard to the observed transgressive segregation, the most accepted explanation is the existence of complementary alleles of additive effects in both parents, with opposite effects within the lines, which differ from the predicted phenotypic frequencies (Rieseberg et al., 1999). One of the predictions of this hypothesis is that complementarity is more easily attained in autogamous populations or inbred lines. This is why when a biparental cross is made, the transgressive individuals are observed in F_2, as demonstrated in this study. This suggests that transgression is positively correlated with the divergence between the parents used in a single-cross hybrid and the heterotic groups to which they belong (Table 1). Even heterosis plays an important role in transgressive expression, and it implies overdominance or at least partial

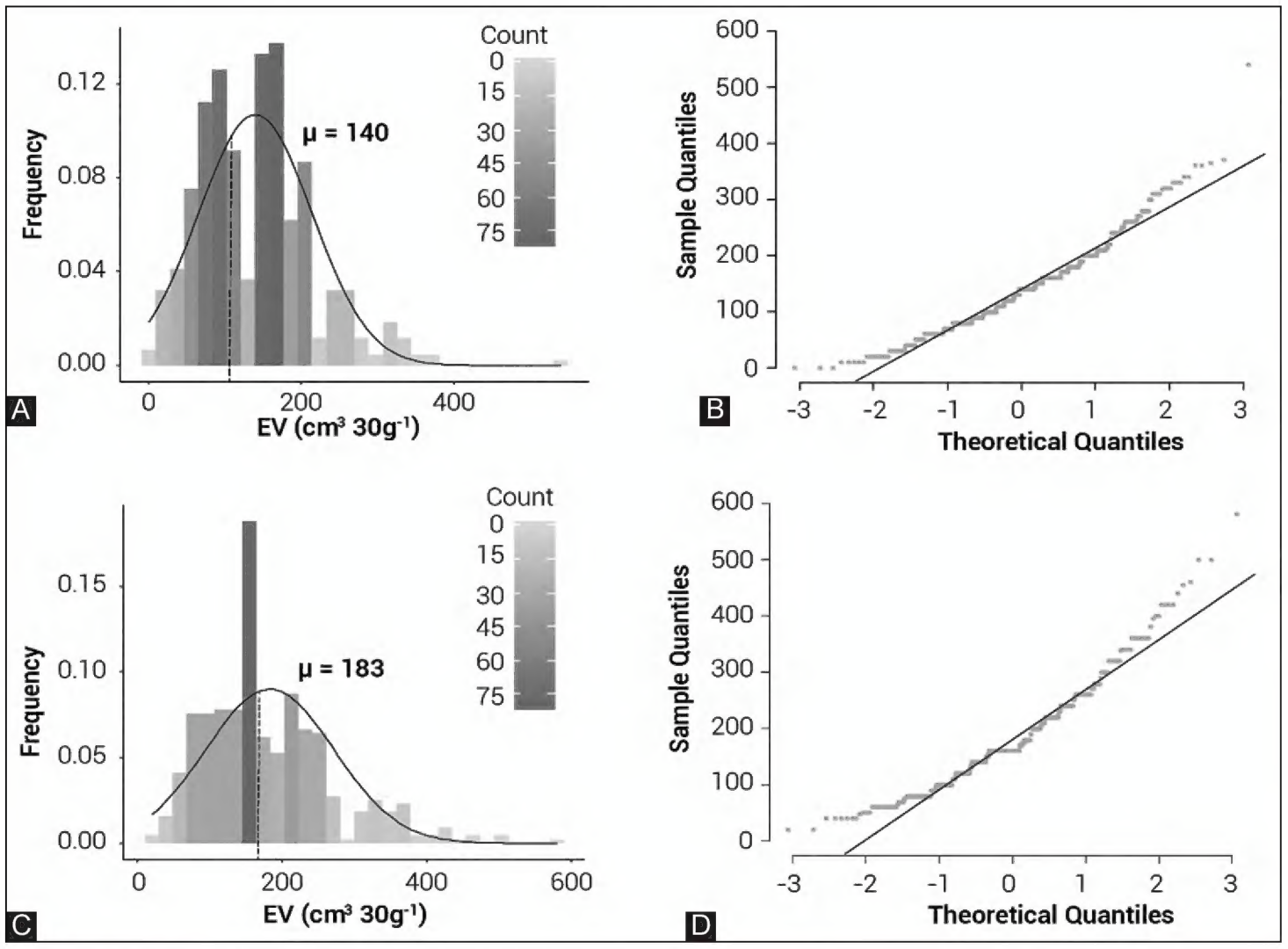

Fig 3. Phenotypic frequencies and Q-Q plot of popping expansion volume evaluated in F_2 populations derived from the crosses between popcorn CML561 and semident CML240 a, b) and CML246 c, d).

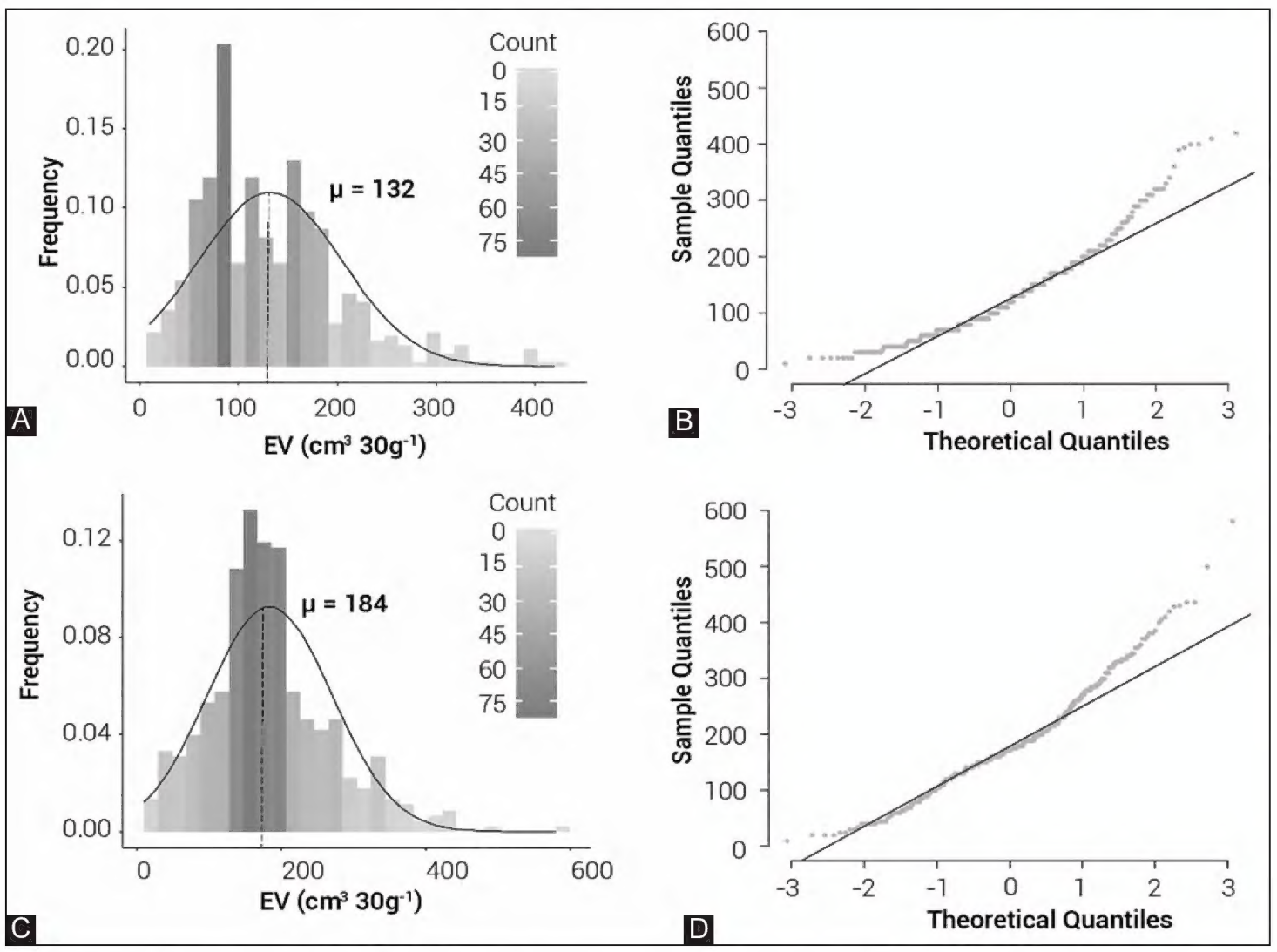

Fig 4. Phenotypic frequencies and Q-Q plot of popping expansion volume evaluated in F_2 populations derived from the crosses between popcorn CML561 and semident CML459 a, b), and CML527 c, d).

dominance at multiple loci in heterozygous individuals (Rieseberg et al., 1999).

Another explanation for the transgressive segregation, in this case, is that possibly all of the lines have some favorable alleles for popping, and the frequency of those alleles is greater in popcorn than in those with other endosperm types. Popping requires that a minimum threshold in the frequency of favorable alleles is reached. The segregants only begin to show popping capacity at relatively high

volumes, because the low frequencies of favorable alleles from the non-popcorn parents in a portion of the segregants in the left tail of the distributions do not reach the threshold for popping. This results in the tail being truncated (Figs 1 to 4), while at the opposite extreme, the phenomenon of meiotic recombination enables the accumulation of favorable alleles in a small fraction of individuals, beyond those possessed by the parental popcorn; thus, inducing transgressive segregation.

Results reported in this study suggest that even though the crosses of popcorn with dent and semident endosperm lines did not exceed the mean EV of the popcorn × flint cross, it is reasonable to suppose that the former also have favorable alleles of additive effects; this is reflected in the presence of transgressive individuals; likewise, it is also assumed that the line with flint endosperm has more favorable alleles that increase EV and when crossing with the popcorn line, those genes confer a gain as well as greater stability in the expression of the trait.

Additional parameters of the distribution

The H test, Lilliefors normality test, Fisher's coefficient of Skewness, and Kurtosis level of the EV evaluated in seven F_2 segregating populations of popcorn × non-popcorn crosses are presented in Table 2. The H test revealed that the popcorn × flint corn cross was statistically higher compared to crosses in which dent and semident endosperm lines were used, indicating that exists a higher frequency of complementary alleles in the flint endosperm with positive effects on EV.

When evaluating the distributions of the populations in relation to the mean and the Lilliefors normality test, it was observed that the EV was not normally distributed and presented positive asymmetry; that is, the weight of the right tail was higher and more distant from the mean. The Kurtosis level showed positive values in most of the endosperm types, which implies that the weight of the tails was greater than in the normal distribution (Figures 1 to 4).

The highest Kurtosis coefficient was found in populations where non-popcorn dent endosperm lines were used as parents, which led to a leptokurtic distribution; that is, with the largest number of individuals at the central region of the distribution, while the lowest coefficient was found in the population from the cross between the popcorn and the flint endosperm lines, indicating a platykurtic distribution, with a low concentration of individuals at the central region, due to the presence of transgressive individuals, which is evident when observing the interquartile distribution.

It is clear that the highest EV values were obtained in the cross where the line with flint endosperm participated as the male, with a higher mean (215 cm^3 $30g^{-1}$) compared to the crosses where lines with semident (160 cm^3 $30g^{-1}$) and dent endosperm (147 cm^3 $30g^{-1}$) participated. Similar results were obtained by Babu et al. (2006) when evaluating a cross of popcorn × flint corn, reporting the presence of 4QTLs with favorable additive effects for EV in the flint endosperm; therefore, populations with flint endosperm could be used in backcrossing or recurrent selection programs (Vieira et al., 2016). On the other hand, Robbins and Ashman (1984) evaluated the relationship between EV and endosperm type in crosses of popcorn × dent and popcorn × flint corn, concluding that both types of crosses presented similar expansion ranges and suggesting that the EV could easily be recovered.

Likewise, the physical and morphological characteristics of the kernel that affect the EV such as the more crystalline disposition of the endosperm, thickness, and a higher degree of fibrillar packing of the pericarp (Da Silva et al, 1993) are more frequently found in the flint endosperm (Hoseney et al., 1983; Park et al., 2000; Gökmen, 2004). In popcorn, the extreme flint corn, the granules of the starch are densely packed, surrounded by protein matrices (Hoseney et al., 1983), and has twice the thermal conductivity as the opaque starch (Da Silva et al., 1993), characteristics which permit to reach a pressure of 135psi inside the kernel allowing to gelatinize and to form flakes

Table 2: Popping expansion volume of the kernels and popping patterns evaluated in seven F_2 segregant progenies

Cross	EV (cm^3 30 g^{-1})		N	L (p-value)	FCs	K
	Median of parents	Mean of F2 progenie				
561 × 240 (SD)	273	139.81 ± 74.62 d	469	7.42E-10	0.91	1.77
561 × 246 (SD)	279	182.39 ± 89.87 b	452	2.20E-16	0.91	1.12
561 × 457 (D)	273	130.39 ± 83.36 e	487	2.20E-16	1.47	2.94
561 × 459 (SD)	268	132.19 ± 72.71 de	522	8.65E-14	1.08	1.52
561 × 461 (D)	278	164.40 ± 85.39 c	547	2.07E-10	1.04	1.72
561 × 527 (SD)	309	183.97 ± 86.07 b	460	5.34E-12	0.79	1.21
561 × 558 (F)	315	215.44 ± 108.76 a	414	2.33E-09	0.70	0.54

EV: popping expansion volume, SD: Semident kernel type, D: Dent kernel type, F: Flint kernel type, N: number of F_2 individuals, L: Lilliefors normality test ($P \leq 0.05$), FCs: Fisher's coefficient of Skewness, K: Kurtosis level. Means±standard deviation for followed by different lowercase letters in the column are significantly different by the H test (*HP≤0.05*).

(Hoseney et al., 1983; Mishra et al., 2014; Sweley et al., 2013; van der Sman and Bows, 2017). All this together could provide an explanation for the higher EV observed in the F2 population derived from crossing with flint endosperm.

The number of genes conditioning EV in the biparental crosses evaluated

Table 3 shows the X^2 and $(1/4)^n$ test of the seven crosses between popcorn × non-popcorns and the estimation of the number of genes involved in popping expansion volume. Under the assumption that the EV is conditioned by major genes with additive effects and that they all have the same effect, the number of genes that defined the EV in the F_2 populations evaluated ranged from three to five, depending on the segregating population.

The popcorn × flint cross was the only one that conforms to the 3-gene model because the popcorn and flint parents probably have some alleles in common, so they cannot be detected since they do not segregate when they are crossed; the highest mean of the F_2 population derived from the popcorn × flint cross supports this hypothesis. These results agree with Babu et al. (2006) who found in a popcorn × flint corn cross that a single major QTL with additive effect explained up to 30 % of the EV phenotypic variance. Similar results were found in a recent study that evaluated the inheritance of EV in popcorn × flint corn crosses. This trait was controlled by a major additive gene in conjunction with polygenic modifiers with both additive and dominant genetic effects and the action of minor genes with dominant effect; such genetic effects may have occurred in the populations with non-flint endosperm evaluated (Coan et al., 2019).

The F_2 populations had an extensive range of EV, even some transgressive individuals (Fig. 1 to 4; Table 3), suggesting additive inheritance of the genes controlling the popping capacity and likely the difference between populations was in the allele frequency of each gene and the type of inter- allelic interaction (Coan et al., 2019). These results agree with Dofing et al. (1991), who found greater additive than dominance effects for EV trait in a popcorn × dent cross. Some Quantitative trait loci (QTL) studies showed similar results in popcorn × dent crosses, in which the additive variance provided by the QTLs ranges from 28 % (Li et al., 2009); to 32-35 % (Li et al., 2006; 2007), 45 % (Lu et al., 2003; Li et al., 2008), 54 % (Li et al., 2007) to 82 % (Dhliwayo, 2008), but for popcorn × flint corn crosses a single major QTL additive effect explained up to 30 % of the phenotypic variance and 4QTL explain the 62 % of the additive variance for popping expansion volume (Babu et al., 2006).

The number of genes estimated agrees with previous reports (Ashman, 1983; Ziegler, 2001; Lu et al., 2003; Babu et al., 2006; Dong et al., 2015; Coan et al., 2019) and indicates that the EV is a trait controlled by few genes, probably with additive effects, which may be an advantage for genetic populations for plant breeding since it would be easy to reestablish the popping capacity in landraces with low expression of this trait such as Mexican native popcorn races. In another hand, there are studies at the genomic level that report candidate genes involved in the synthesis of starch polysaccharides, linoleic acid, amylose, α-zeins, lipids, and cell wall components related to improving the popping capacity; such relationships differ between the types of endosperm in corn (Borras et al., 2006; Paes et al., 2016; Vázquez-Carrillo et al., 2019), which suggests that the genes involved could have pleiotropic effects on morphological characteristics of the kernel and the endosperm and suggest a gradual indirect selection on these biochemical compounds, which could explain the high heritability of the popping capacity (0.62 to 0.96) in popcorn (Ziegler, 2001). Results from a recent investigation in which an EigenGWAS was carried out in 526 CMLs showed that 62 significant loci were undergone a gradual selection and the allele frequencies for related SNPs that promote popping was 55.27 %; in other words, non-popcorn CMLs still contain standing variation for alleles that promote popping (Li et al., 2021). The above could

Table 3: Individuals per population and popping phenotypic frequencies expected and observed in seven F_2 segregant progenies derived by crosses between popcorn×non-popcorn inbred lines into three classification groups

Cross	G1			G2		G3		X^2C	[1]Genes	$(1/4)^n$	[2]Genes
	N	E	O	E	O	E	O				
561 × 240 (SD)	469	1	0	1	1	357	350	1.1	5	4.44	4
561 × 246 (SD)	452	0	1	0	1	344	376	4.7	5	4.41	4
561 × 457 (D)	487	2	2	2	1	331	314	1.4	4	4.46	4
561 × 459 (SD)	522	1	0	1	1	398	398	1.0	5	4.51	5
561 × 461 (D)	547	1	1	1	2	417	426	5.2	5	4.04	4
561 × 527 (SD)	460	2	0	2	3	313	283	4.8	4	3.63	4
561 × 558 (F)	414	7	3	7	10	233	237	3.8	3	2.68	3

SD: Semident kernel type; D: Dent kernel type, F: Flint kernel type, N: Number of F2 individuals, E: Expected absolute frequency, O: Observed absolute frequency. G1: homozygous individuals with the same EV as P1; G2: homozygous individuals with the same EV as P2; G3: heterozygous individuals with intermediate EV; two degrees of freedom; $X^2_{t_0=0.05}$=5.991. [1]Genes: Estimated genes using the X^2 test; [2]Genes: Estimated genes using the $(1/4)^n$ test.

explain the VE segregation patterns and the transgressive individuals present in the seven F2 populations studied in this work, as well as the variation in the number of genes involved in the EV trait.

Results of this research constitute a contribution towards the search for genomic regions associated with popping capacity in a more precise way with high-density markers, aimed at establishing a scheme of marker-assisted selection for popping capacity in popcorn.

CONCLUSIONS

The segregating population derived from the biparental cross of popcorn × flint, that is, the population derived from CML561 x CML558 lines, was the most divergent population, with the most symmetrical distribution, and therefore, the most appropriate for molecular studies associated with popping capacity in popcorn. Results indicated the existence of three to five major genes related to popping expansion volume. Transgressive segregation was present in all populations, induced by meiotic recombination, thus making it possible the combination of alleles showing F2 individuals whit popping expansion either higher or lower than the parental ones, which supports the hypothesis that non-popcorn lines still contain standing variation for alleles that promote popping.

AUTHORS' CONTRIBUTIONS

Viridiana Trejo-Pastor and Amalio Santacruz-Varela conceptualized and designed the work, planned and carried out the field experiment, analyzed the results, and wrote the manuscript. Denise E. Costich directed the biparental crosses at the CIMMYT experimental station located at Toluca, Estado de Mexico, Mexico. Roberto de la Cruz Díaz-Juárez carried out the field experiment. Higinio López-Sánchez and Leobigildo Córdova-Téllez provided the necessary guidelines and critically contributed reviewing the manuscript.

DECLARATIONS OF INTEREST

The authors declare that they have no conflicts of interest.

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